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ELECTRICAL ACTIVITY AND BEHAVIOR IN THE SOLITARY HYDROID *CORYMORPHA PALMA*. II. CONDUCTING SYSTEMS

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Attempts to understand the relationship between various types of cnidarian conducting systems and between electrical activity in these systems and behavior have been based largely on three general approaches; (1) surgical alteration of the system, (2) application of drugs or alteration of the ionic environment, and (3) electrical stimulation. The use of these methods on hydrozoan and scyphozoan medusae and on anthozoans has resulted in considerable advances in understanding the physiological basis of their behavior. However, with the exception of *Hydra* it has not been possible to apply all of these techniques to individuals of any one species of hydrozoan polyp because of small size and the presence of a perisarc. We therefore decided to apply these techniques to *Corymorpha*, which, because of its large size and reduced perisarc, appeared well suited to an investigation utilizing all of these approaches.

Electrical and behavioral responses to electrical stimulation have been examined in the hydroids *Hydractinia* (Josephson, 1961) *Cordylophora* (Mackie, 1968), *Tubularia* (Josephson, 1961, 1965; Josephson and Uhrich, 1969) *Hydra* (Josephson, 1967; Josephson and Macklin, 1967, 1969; Kass-Simon, 1972) and *Obelia* (Morin & Cooke, 1971). In *Tubularia* Josephson (1965) found evidence for three conducting systems: (1) a distal opener system producing a small pulse conducted at 15 cm/sec and associated with a downward flaring of the distal tentacles; (2) a triggering system which triggers the type of pulses that normally appear spontaneously, conducts at approximately 17 cm/sec, and has no electrical correlate; and (3) a labile slow system producing propagated potentials which travel at about 6 cm/sec in the stalk.

There is a long history of investigations involving the application of drugs and ions to medusae (see Mackie and Passano, 1968), while somewhat less work has been done on anthozoans (see Ross, 1960a, 1960b), and relatively little on hydrozoan polyps. Parmentier and Case (1973, page 17), working on the hydroid *Tubularia* found that: "The sympathomimetic compound ephedrine induces steady rates of impulse production in the spontaneous pulse system regardless of the pre-treatment discharge pattern without disrupting behavioral responses and coordinated epitheliomuscular output." From these results they concluded that the epithelial system had little direct relationship to localized behavioral activation, although they felt that it might serve to coordinate more symmetrical output patterns

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throughout the organism. In view of these results we decided to test the effect of ephedrine and related sympathomimetic drugs on electrical activity and behavior in *Corymorpha*.

Bullock (1943), investigating the effects of alteration in the ionic environment on the neuromuscular systems of medusae, found that excess Mg^{++} first depresses myo-neural junctional facilitation and then neuro-neural junctional facilitation. In *Tubularia* Josephson (1965) was able to separate electrically triggered distal opening system pulses (DOSP) from the following slow wave by application of excess Mg^{++}. He interpreted this result as indicating that the DOSP was associated with nerve activity while the slow wave was a muscle action potential, with excess Mg^{++} blocking the link between the two. These results led us to the idea for the Mg^{++} bridge experiment described in this paper.

Mackie (1968) made the interesting discovery that tetrodotoxin, which selectively interferes with the early transient sodium-conductance increase in lobster (Narahashi, Moore, and Scott, 1964) and squid axons (Nakamura, Nakajima, and Grundfest, 1965), had no effect on electrical activity or behavior of *Cordylophora* over periods of more than an hour. This finding, if generally applicable to coelenterates, is of considerable interest since it may indicate that their pacemakers and conducting systems differ from those of most organisms by not being Na^{+} dependent. This experiment was therefore repeated on *Corymorpha*.

Materials and Methods

Electrical stimulation

In the majority of the experiments involving electrical stimulation a *Corymorpha* was laid out horizontally on a sheet of cork and held in place by Pt-Ir staples across the stalk. Stimulation was normally basal; but stimuli were also applied near the neck, or at both ends of the stalk, either simultaneously or with varying interstimulus delays. Animals standing upright on sand were also stimulated to establish whether the position of the animal or the restraint of the staples produced any effect on response to stimulation.

Stimuli were applied from Grass S4 and S48 stimulators through Grass Stimulus Isolation Units. The stimulating electrodes used in most experiments were either Pt-Ir (0.2 mm) or Ag-AgCl (1.6 mm) insulated to the tips with "Insulex" or silicon rubber. In a few experiments a "Tygon" suction electrode (i.d. 1.6 mm), similar to those used for recording, was used for stimulation with a Pt-Ir indifferent electrode in the bath. Electrode polarity was periodically reversed to avoid polarization. Unless otherwise noted stimuli were 5 msec monophasic square waves of varying voltages. The pulse duration was chosen arbitrarily because it appeared to be adequate to obtain a full response from most animals. Stimulation was increased from low voltages fairly rapidly until an electrical or behavioral threshold was reached.

Unless otherwise specified recording electrodes, conditions and equipment were as described in the previous paper. Interelectrode distances were measured using dividers or a small ruler. When these distances changed noticeably during the course of a burst the distances before and after stimulation were averaged. Measurements were made often enough so that the variability introduced by averaging

was very small compared to the apparent normal variability of the system. Methodological details of some further experiments are discussed in conjunction with the results.

Drugs

All drug solutions were made up in filtered sea water (5 μ pore size filter) with the final pH adjusted within the range of pH 7.0–8.0. All experiments were run at $20 \pm 1°$ C. Drug concentrations and numbers of replicates are summarized in Table I. Several types of experimental chambers were used, with the exact size and shape depending on the size of the preparation and on the details of the experiment. Solutions were withdrawn and replaced using two syringes with a capacity greater than that of the bath. Immediately preceding solution changes the electrodes, and the tissue to which they were attached, were lowered as near to the bottom of the bath as possible, thus considerably reducing the chances of the electrode detaching during the change of solutions. The fluid to be replaced was then withdrawn almost completely, resulting in only a slight dilution of the fluid being added. The general procedure used in these experiments was (a) to record from the preparation in sea water for a minimum of 15–20 minutes, (b) to apply the drug being tested and record for 15–20 minutes, then (c) to replace the drug with sea water and record another 15–20 minutes.

For an experiment involving tetrodotoxin a sheet of stalk tissue was arranged on a thick pad of porous cloth in an effort to provide the drug with free access to both sides of the sheet. Due to the low level of spontaneous activity in stalk tissue, the response to a single electrical stimulus given every 100 seconds during periods of observation was used as an additional assay for effects of the drug.

Inorganic ions

Experiments involving altered ionic composition of the bathing solutions included (a) application of Moore's Ca^{++}-free sea water (Cavanaugh, 1956) which has the following ionic composition (g/liter), NaCl, 25.48; KCl, 0.72; $MgCl_2 \cdot 6H_2O$, 6.94; $MgSO_4 \cdot 7H_2O$, 4.11; and (b) application of various concentrations of excess Mg^{++} in the form of $MgCl_2$ solutions. For some of the experiments on the effects of $MgCl_2$ the isolated stalk of a large *Corymorpha* was draped across three compartments cut into a block of wax and held in place by staples passing over the stalk. There was some leakage of solutions along the stalk over the partitions separating the compartments, but this leakage had no apparent effect during the relatively short time required for the experiments. A suction electrode was used to record from the portion of the stalk in each compartment, and stimulation was via two wires placed on opposite sides of the base of the stalk. A precondition for proceeding with the experiment was that five successive stimuli at 1-minute intervals had to produce one or more pulses recorded on each of the electrodes. Once this precondition had been met, the sea water in the center compartment was replaced with isotonic $MgCl_2$ and stimulation at 1-minute intervals was continued. The length of time that the $MgCl_2$ remained in the central chamber depended on the responses of the individual animal; but the $MgCl_2$ was eventually replaced by repeated changes of sea water. The sea water in the first and third

FIGURE 1. Some characteristic responses of the triggered pulse system in *Corymorpha*. (A and B.) Both records, from two different animals, show consecutive pulses, the first spontaneous and the others triggered. Note that in A the pulses appear similar although they are traveling in opposite directions; (C and D.) effect of increased stimulating voltage on electrical response. Sections were removed from some of the records (dotted lines) to allow reproduction. The large S and arrow adjacent to each diagram of *Corymorpha* in this and all other figures in this paper indicate the point at which the stimulus was applied. Stimulus artifacts in the records in this and all other figures in this paper are marked with upward-pointing arrows. Vertical scales of A and B are 500 μV; those of C and D are 50 μV. Horizontal scale equals 0.1 sec. Portions of the records were retouched for reproduction.

chambers was also replaced, due to some leakage of $MgCl_2$ from the center compartment into the others. Single stimuli were then given at irregular intervals to observe the progress of recovery from $MgCl_2$.

RESULTS

Behavioral responses of the unrestrained animal.

The responses to stimulation of a proximal tentacle of an unrestrained animal with successive single pulses of increasing voltage are similar to those described by Parker (1917): first only the stimulated tentacle responds; then adjacent tentacles; then the distal tentacles and the proboscis, which may bend toward the stimulated tentacle; and finally a stalk contraction may be produced.

Electrical and behavioral responses of the restrained animal.

Characteristics of the triggered pulse system (TPS) in response to stimuli of differing voltages. The pulses most commonly generated in the stalk in response to electrical stimulation appear to be identical to spontaneously occurring pulses (Fig. 1A, B). However, to facilitate discussion triggered pulses will be called TPs and the system will be called the TPS (triggered pulse system). Frequently, especially at low stimulating voltages, the electrical response to stimulation is a

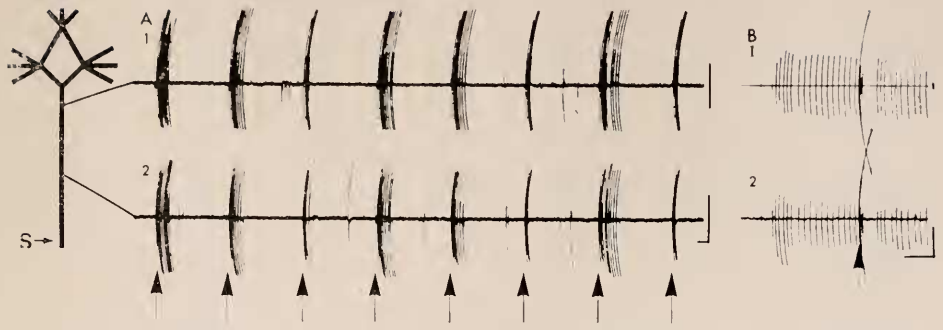


FIGURE 2. Excitation and inhibition produced by single electrical stimuli; (A.) intact animal, (B.) a sheet of stalk tissue. Vertical scale equals 100 μV; horizontal scale equals 5 sec.

single small pulse travelling along the stalk in a non-decrementing fashion (Fig. 1C). In the majority of cases where this is the sole electrical response there is either no visible reaction or there is a slight overall contraction.

At slightly higher stimulating voltages a burst of pulses is usually produced (Fig. 1D). Associated with this burst there is usually a more violent contraction and there may be a simultaneous oral flexion of all proximal tentacles sometimes accompanied by an aboral flaring of the distal tentacles. Some animals show a fairly clear-cut threshold for burst initiation, while in others this threshold varies. Both the intensity of contraction and the length of a stimulus-initiated burst appear to depend on the strength of the stimulus. Stimulation normally produces bursts of electrical activity (Fig. 2A); but it can also interrupt ongoing spontaneous electrical activity (Fig. 2B). Fig. 3 shows several bursts all produced by the same animal in response to single electrical stimuli.

Conduction velocity in the TPS. Apparent conduction velocity in the TPS varies both within an animal over time and, to an even greater extent, between animals. There is no apparent difference in the conduction velocity as measured between two points on the stalk and between one point on the stalk and another on the hydranth. The average conduction velocity between two points on the stalk determined from a minimum of five measurements on each of ten animals was 15.7 cm/sec. Individual averages varied from 10.9 cm/sec to 21.2 cm/sec. This average value is quite close to the average spontaneous pulse conduction velocity of 15.9 cm/sec which appears to strengthen the case for the identity of these two systems. However, the use of average values may be misleading. Records sufficient to allow calculation of conduction velocities for both triggered and spontaneous pulses in the same preparation were obtained from six animals. In four of these there was little difference ($\pm$1 cm/sec) in velocity. The other two gave the following averages for consecutive sets of spontaneous and triggered bursts. (1) spontaneous 23.8 cm/sec, triggered 16.9 cm/sec, spontaneous 26.2 cm/sec; (2) spontaneous 14.0 cm/sec, triggered 10.5 cm/sec, spontaneous 14.0 cm/sec.

Tests for polarization in the TPS. To check whether the TPS was polarized, the stimulating electrodes were placed in the neck region of a *Corymorpha* with the recording electrodes farther down the stalk. The average conduction velocity

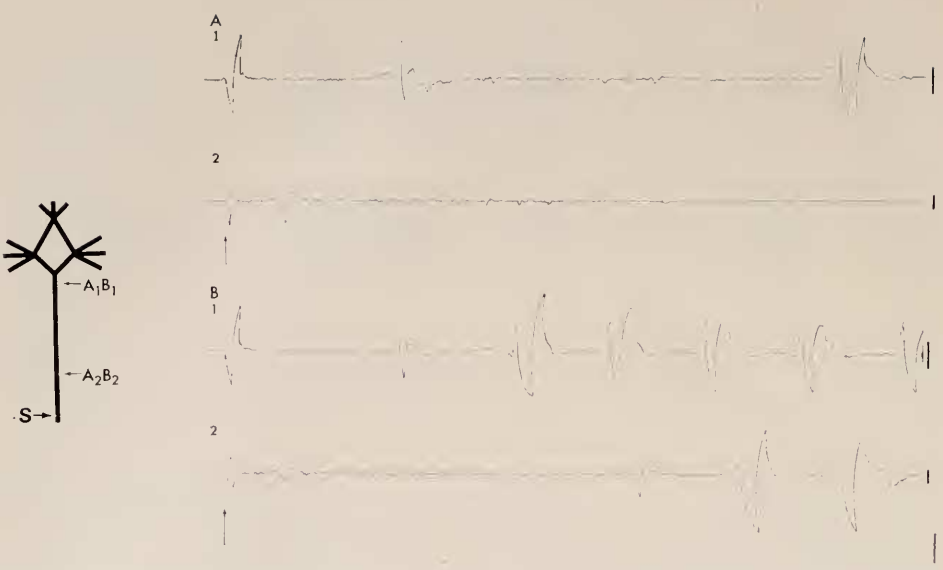


FIGURE 3. Variability in pulse patterns of one *Corymorpha* in response to single electrical stimuli; (A.) large pulses interspersed among a series of much smaller pulses; (B.) a single triggered pulse traveling distally has apparently initiated a burst of activity originating from the other end of the stalk. The significance of the apparent differences in initial polarity of large and small pulses is unknown. Stimulating and recording electrodes were in the same positions for each record. Stimulus artifacts in the records are marked with upward-pointing arrows. Vertical scale equals 50 μV; horizontal scale equals 0.1 sec.

determined from a minimum of five measurements on each of eight animals was 15.2 cm/sec. Individual averages varied from 11.5 cm/sec to 19.3 cm/sec. From these data it appears the TPS is non-polarized, although the possibility of normal input polarization is not eliminated by these experiments since electrical stimuli would be expected to activate the conducting system directly. Experiments in which stimulating electrodes were placed at the base and neck, with three recording electrodes between, show very similar-appearing pulses traveling base to neck and neck to base, respectively.

Possible facilitation in the TPS. Some records have been obtained which might be interpreted as showing facilitation (Fig. 4). However, since the recordings were made with suction electrodes it is difficult to evaluate these results because the relation of the electrode to the underlying tissue and the strength of the suction can change over time as the animal expands and contracts.

The distal opening response. Torrey (1904) and Parker (1919) describe the distal opening response in *Corymorpha palma,* and both Wyman (1965) and Krasilovsky (1967, unpublished) apparently had little trouble eliciting it in *Corymorpha palma* and *C. pendula,* respectively. In the present study, on the contrary, consistent distal opening was obtained in only two of more than forty animals tested over the period of approximately a year, with a few other animals giving an inconsistent response. Since a majority of the specimens of *Tubularia*

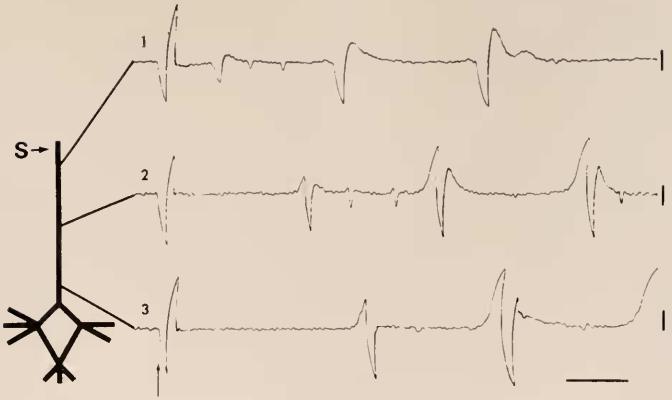


FIGURE 4. Example of possible facilitation during a triggered burst in *Corymorpha*. Vertical scale equals 50 μV; horizontal scale equals 0.1 sec.

tested gave a distal opening response with a similar stimulating set-up, the difficulty encountered in obtaining this response has yet to be explained.

In two specimens of *Corymorpha* distal opening was sometimes obtained without any other observable response. In one case the response was associated with a burst of large pulses; in the other there was a single small pulse (Fig. 5). In those animals where there is a burst of large pulses associated with the distal opening response, it is often impossible to distinguish between bursts which are associated with distal opening and those which are not.

Several lines of evidence point to the possible existence of more than one pulse system. First, there are often two fairly distinct sizes of pulses (Fig. 6A), or pulses of two distinct waveforms (Fig. 6B). Secondly, several records appear to show differing conduction velocities for large and small pulses (Fig. 6 C, D).

Alterations in conduction velocity in response to continued stimultion. Most animals showed only a slight decrease in conduction velocity following long-con-

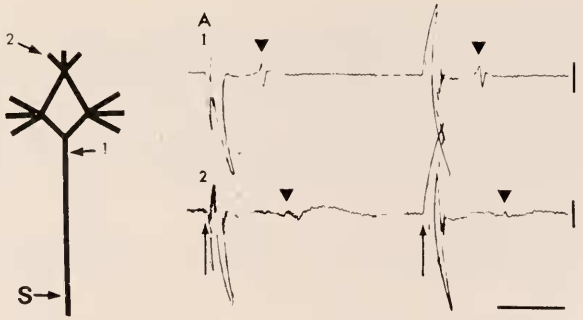


FIGURE 5. The single small pulse (marked by a triangle) associated with distal opening in the absence of any other observable reaction, as recorded from the stalk and distal tentacle of a *Corymorpha*. Portions of the records were retouched for reproduction. Vertical scale equals 200 μV; horizontal scale equals 0.1 sec.

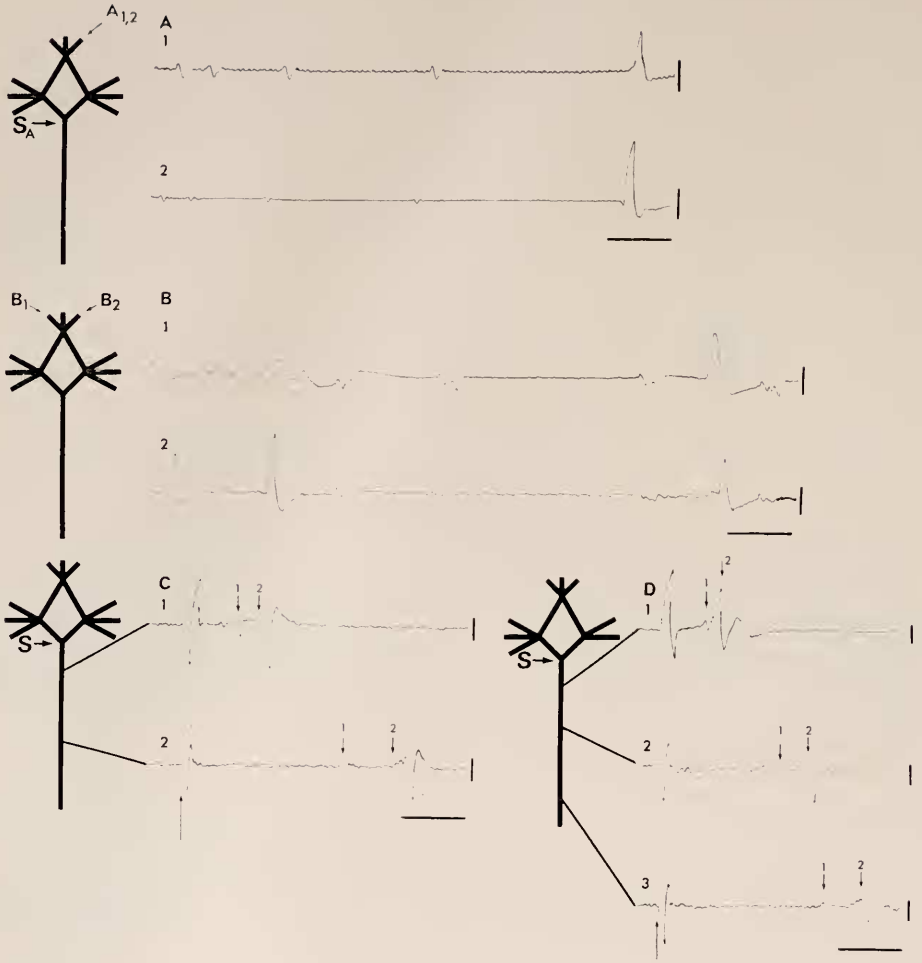


FIGURE 6. Evidence for two conducting systems in *Corymorpha;* (A.) pulses of two distinct sizes; (B.) two types of pulses differing in waveform as well as size. These pulses were triggered, but location of the stimulating electrode was not recorded; (C and D.) evidence for two distinct pulse sizes and conduction velocities in the stalks of two different animals. The two pulses in each record are indicated by numbers and arrows. Conduction velocities of the large and small pulses are, respectively, 13.9 and 17.7 cm/sec in C, and 16.8 and 19.7 cm/sec in D. The vertical scale in A and B equals 200 μV except A2 which is 1 mV, and vertical scale in C and D equals 50 μV; horizontal scale in all records equals 0.1 sec.

tinued stimulation, but in several cases the apparent velocity dropped to about half the original velocity.

Investigations on the layer of origin of triggered stalk pulses

The protocol of the abrasion experiments, which were used to establish the tissue of origin of triggered stalk pulses, can best be discussed together with the

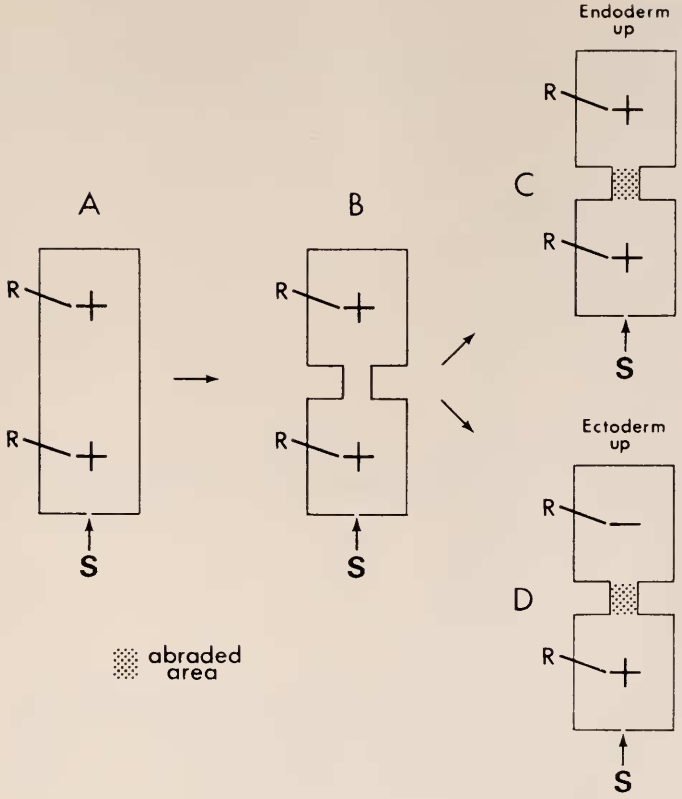


FIGURE 7. Diagrammatic representation of experiment establishing the tissue of origin of triggered stalk pulses in *Corymorpha*. See text for details. S and adjacent arrow indicate the point at which stimuli were applied; R indicates a recording electrode; + indicates the presence of a triggered pulse; — indicates its absence.

results and by reference to Figure 7. A stalk of *Corymorpha* was split longitudinally and pinned out as a sheet of tissue. In three cases this sheet was endoderm up and in three cases ectoderm up. A stimulating electrode was placed at one end and two recording electrodes were placed along what was formerly the long axis of the stalk. Five successive single stimuli were given at 1-minute intervals and either a single pulse or a burst of pulses was recorded on both electrodes in response to each stimulus (Fig. 7A). Transverse incisions were then made in the stalk tissue between the two recording electrodes leaving them connected by a narrow tissue bridge, and five single stimuli were again given at 1-minute intervals with the response being recorded on both electrodes (Fig. 7B). The upper layer of the tissue forming the bridge between the two recording electrodes was then abraded away with a glass needle. The operation could be performed with minimal injury to the underlying tissue because of the remarkably tough mesoglea of *Corymorpha*. When the endodermal side of the bridge was abraded (Fig. 7C)

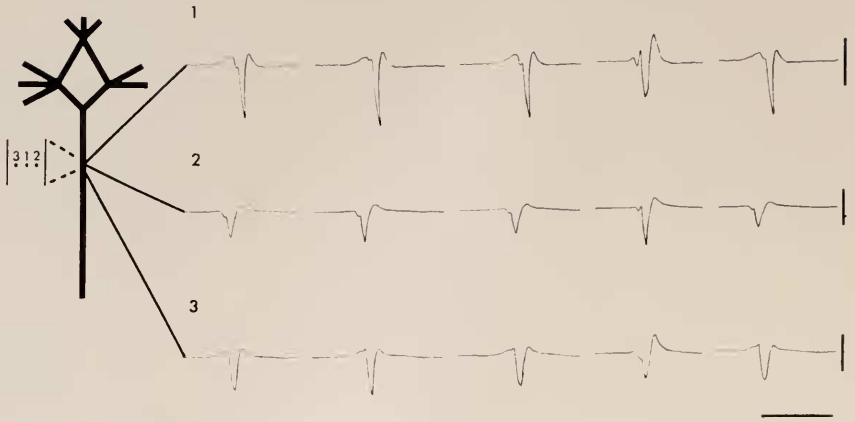


FIGURE 8. Records from three electrodes lined up transversely across the stalk of a *Corymorpha*. Vertical scale equals 500 μV; horizontal scale equals 0.1 sec.

the pulses continued to pass across the bridge, but they failed to do so when the ectoderm was abraded (Fig. 7D).

Investigations of the spatial distribution of the TPS

Localized conduction tracts were sought by recording with three suction electrodes lined up at approximately 1 mm intervals transversely across the stalk of each of two specimens of *Corymorpha*. Records obtained from all of the electrodes were relatively similar in time of arrival, waveform, and amplitude (Fig. 8).

In a further effort to detect signs of preferential conduction an experiment of Parker (1919), which is illustrated in Figure 9, was repeated while recording from three suction electrodes placed at various points on the two arms of the preparation. Recordings were made from 3 replicates of each of the three types of preparation. In all three types typical triggered pulses were recorded from both arms following

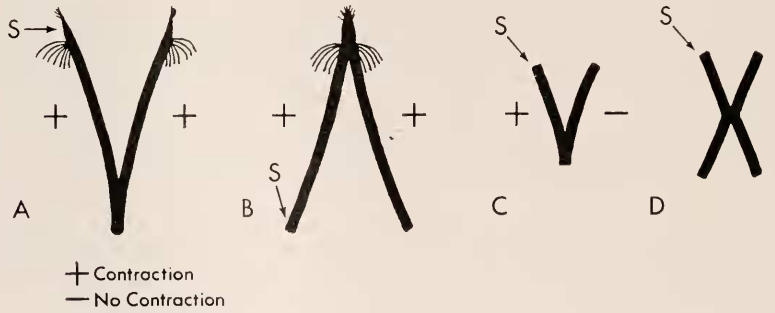


FIGURE 9. Diagrammatic summary of some of Parker's (1919) experiments on conduction pathways in *Corymorpha*. A–C give Parker's results. D is a modification of C used in the present experiments in which the hydranth was removed and the two arms of the preparation remained connected by a bridge of stalk tissue.

TABLE I

Summary of experiments with drugs and ions

Compound	Concentration	Preparation	Replicates	Effect
				I. *Drugs*
Tyramine HCl	0.01 M	Whole animal	3	General increase in activity as well as an increase in bursting tendency which was especially noticeable in the proboscis
Tyramine HCl	0.01 M	Excised hydranth	3	General increase in electrical activity with an increase in bursting tendency which was especially noticeable in the proboscis (Fig 11).
Tyramine HCl	0.01 M	Excised proximal tentacles	3	General increase in electrical activity (3 PTs) and in bursting tendecy (2 PTs).
D-Amphetamine SO_4	0.01 M	Excised hydranth	3	No clear change in electrical activity.
Ephedrine HCl	0.01 M	Whole animal	6	Pulse size was generally reduced. Some parts of the animal showed increased electrical activity, while that in other parts was reduced. No consistent pattern of change was apparent.
Ephedrine HCl	0.005 M	Whole animal	3	Two animals showed a clear increase in electrical activity; the third showed little change.
Ephedrine HCl	0.01 M	Excised hydranth	4	A fairly consistent pattern with the proximal tentacles showing a burst immediately on application of the drug, then becoming almost totally inactive, while proboscis activity changed almost entirely to regular, rather sharp bursts with correlated concert activity (Fig. 12A).
Ephedrine HCl	0.01 M	Excised proximal tentacles	9	Seven of nine tentacles showed a considerable increase in pulse activity (Fig. 12B).
Phenylpropanolamine	0.01 M	Whole animal	3	Tremendous increase in electrical activity in one animal (Fig. 13A), a slight increase in another, and no apparent change in a third.
Phenylpropanolamine	0.01 M	Excised hydranth	3	Considerable increase in electrical activity with all pulses grouped into bursts (Fig. 13B).
Phenylpropanolamine	0.01 M	Excised proximal tentacles	3	Activity increased to a steady stream of pulses.
Tetrodotoxin	10^{-5} g/ml	Excised hydranth	2	No change in spontaneous electrical activity within 60 minutes in one case or 195 minutes in the other.
Tetrodotoxin	10^{-5} g/ml	Sheet of stalk tissue	1	No effect on either spontaneous or stimulus-induced pulses within 312 minutes.

TABLE I—(*Continued*)

Compound	Concentration	Preparation	Replicates	Effect
				II. *Inorganic ions*
$MgCl_2$	Isotonic (0.54 M)	Whole animal	3	Pulses were eliminated, but were restored when the animal was returned to sea water.
$MgCl_2$	1 part isotonic: 9 parts sea water	Whole animal	3	A definite slowing of electrical activity in two cases; no effect in the other.
Ca^{++}-free Sea Water	Isotonic	Whole animal	3	Pulses became smaller and less frequent. Animals assumed a frozen appearance without showing any obvious relaxation.

stimulation of one. In the preparation with the two arms joined basally (Fig. 9A) the perisarc was slipped off and a recording electrode was placed near the base. There was clear conduction through the basal portion of the stalk as evidenced

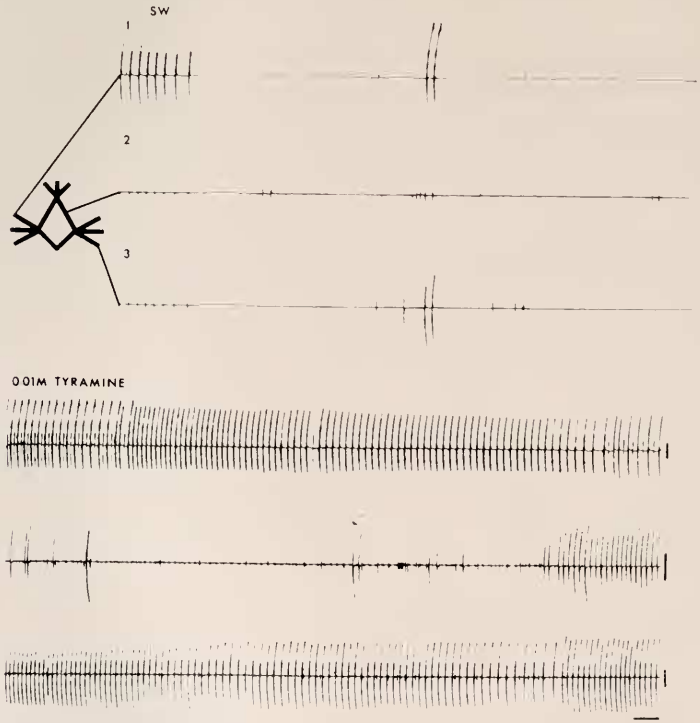


FIGURE 10. Effect of 0.01 M tyramine HCl on electrical activity in an excised hydranth. Record is continuous except that the amplifiers were turned off during the change of solutions. Vertical scale equals 1 mV; horizontal scale equals 5 sec.

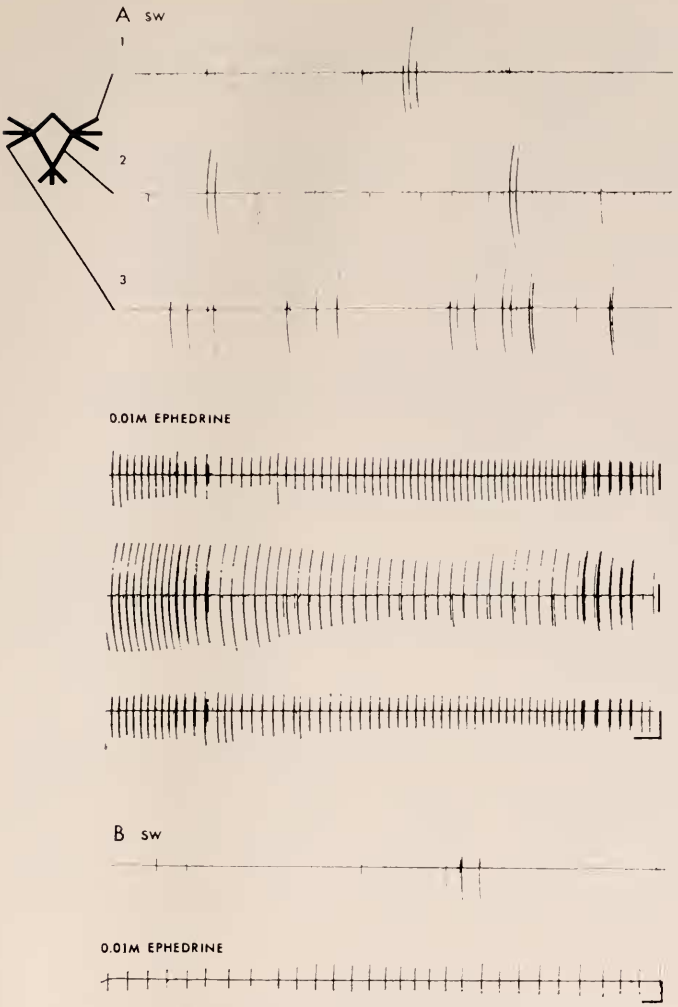


FIGURE 11. Effect of 0.01 M ephedrine HCl on electrical activity of excised parts of *Corymorpha;* (A.) an excised hydranth; (B.) an excised proximal tentacle. Records are continuous except that the amplifiers were turned off during the change of solutions. Vertical scale equals 1 mV; horizontal scale equals 5 sec.

by large pulses in both arms of the preparation; but within the area normally covered with perisarc the pulses were greatly reduced in amplitude.

Experiments involving drugs

Most of the results are summarized in Table I and Figures 10 through 13. Some of the findings presented in Table I are discussed more extensively below along with those results which could not be adequately presented in tabular form.

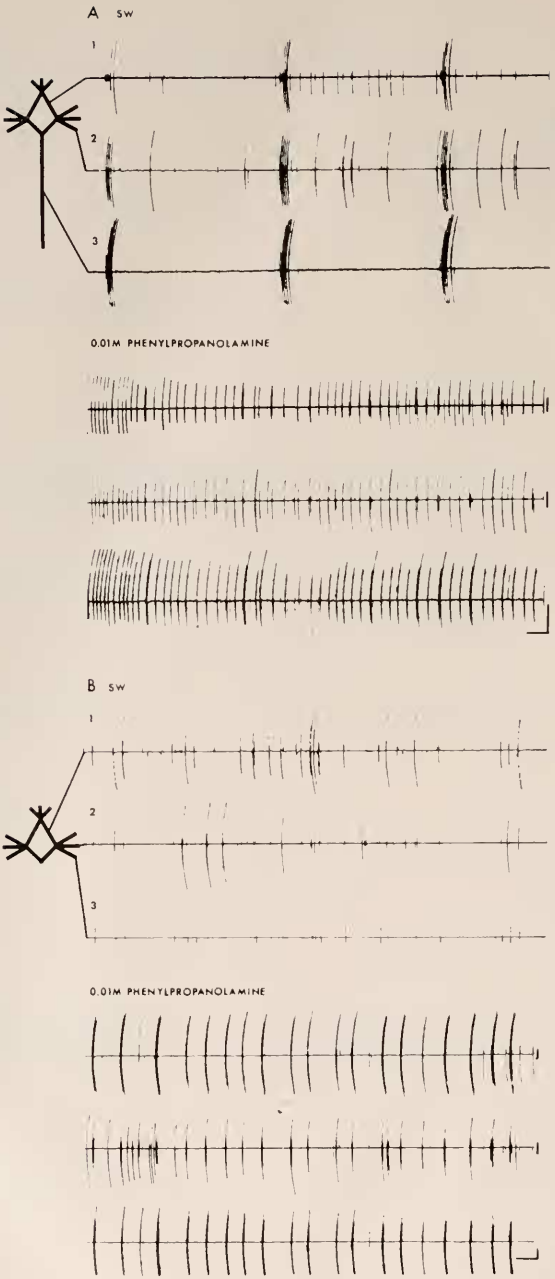


FIGURE 12. Effect of 0.01 M phenylpropanolamine on electrical activity of an intact *Corymorpha* and of excised parts; (A.) an intact animal, record continuous; (B) an excised hydranth. Record shows activity approximately one minute after drug application. Vertical scale equals 500 μV; horizontal scale equals 5 sec.

Ca^{++}-free sea water had rapid and clear-cut effects on electrical and muscular activity. Although there was no apparent muscular relaxation almost all motion ceased within 15 minutes in each of three animals which were placed in Ca^{++}-free sea water. Pulse amplitude generally became smaller and pulses less frequent, although they continued long after all motion had disappeared. Following reapplication of sea water the distal tentacles were in motion within 30 seconds even when they had been in Ca^{++}-free sea water for up to 270 minutes. Within 5 minutes the proximal tentacles and proboscis were also back in motion.

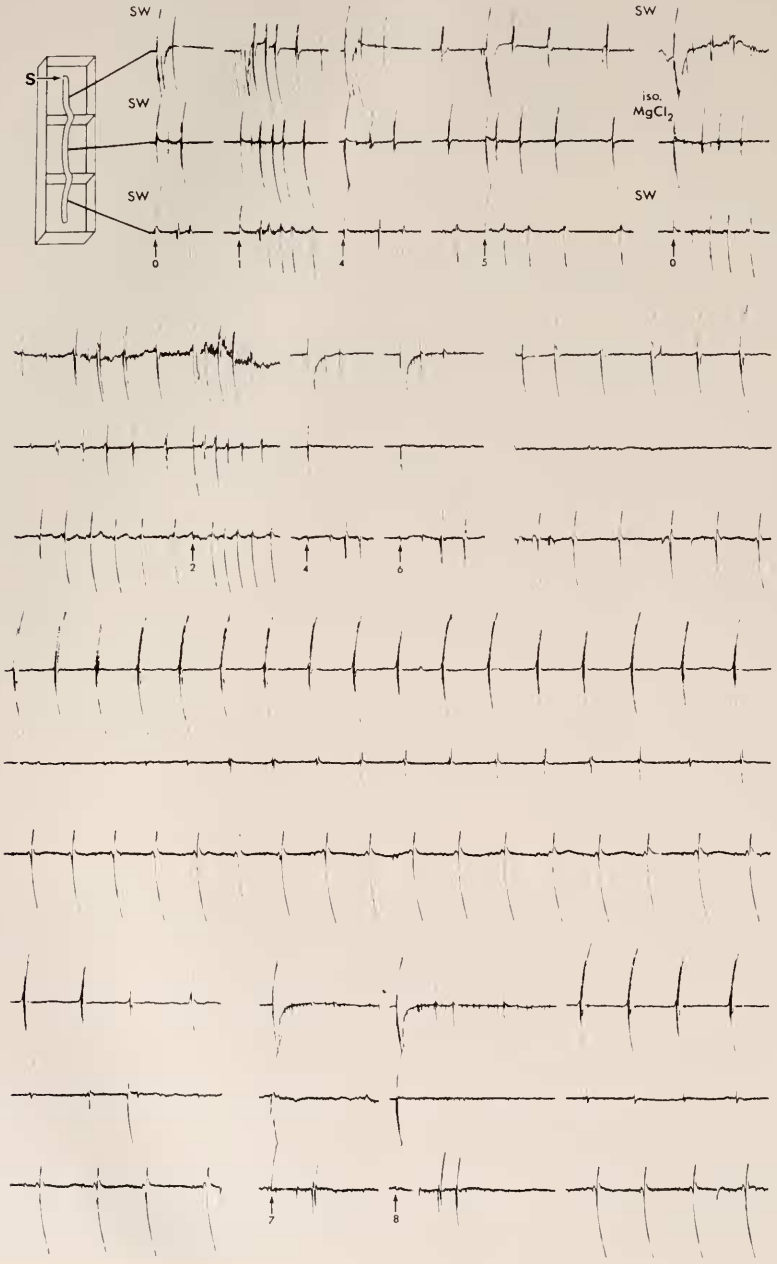
All five of the isolated stalks which were run in the three-chamber bath with central $MgCl_2$ substitution gave essentially the same results, although in several instances complete recovery of the large pulses in the middle chamber following return to sea water was not obtained. Electrode slippage sometimes made repositioning necessary. In general, the pattern was that shown in Figure 13, with little change in the pulse pattern in response to stimulation for at least the first 2 minutes after the addition of $MgCl_2$ to the middle chamber. Shortly thereafter the electrical response of the portion of the stalk in the middle chamber began to decrease until it eventually disappeared, as shown in Figure 13 at minutes 4 and 6 after the addition of $MgCl_2$. Conduction persisted through the portion of the stalk in this compartment, however, as evidenced by continuing full-sized pulses in compartments 1 and 3. The record shown in Figure 13 is unusual in that when the recorder was turned on preparatory to giving the seventh stimulus following addition of $MgCl_2$ a spontaneous burst was found to be in progress, as evidenced by the large pulses recorded in chambers 1 and 3. For approximately the first half of the burst there was no response from the portion of the stalk in $MgCl_2$. Then a small pulse began to appear which grew in amplitude with successive pulses of the burst. Once the portion of the stalk in the central chamber had repeatedly failed to respond to electrical shocks (at minutes 7 and 8 after addition of $MgCl_2$ in Figure 13) the $MgCl_2$ in the central chamber was replaced with sea water. Stimulation was then continued at random intervals to check the recovery of large pulses in the central chamber. Recovery usually occurred (minutes 48 and 49 after return to sea water in Figure 13), although in several cases the pulses failed to regain their original amplitude.

Discussion

Several of the differences between the spontaneous pulse systems and conducting systems of *Corymorpha* and *Tubularia* can be attributed to the presence of well-developed epitheliomuscular cells in the stalk of *Corymorpha*. The stalk of *Tubularia,* by comparison, is covered with perisarc and the muscle cells beneath this are apparently greatly reduced or absent (Hyman, 1940). Functionally *Corymorpha* can be regarded as a *Tubularia* with an elongate neck.

The triggered pulses produced in both the stalk of *Corymorpha* and the neck of *Tubularia* appear to be identical to pulses spontaneously originating in these areas. Originally no electrical correlate of the triggering system had been recorded in the stalk of *Tubularia,* but R. K. Josephson (University of California, Irvine, personal communication) has recently recorded small labile pulses associated with this system. This is again similar to the situation in *Corymorpha* where pulses are greatly reduced in amplitude when passing through the perisarc-covered base. On the basis

of these findings there appears to be a clear association between the presence of a well-developed musculature and the generation of the large electrical pulses discussed in this and the preceeding paper.



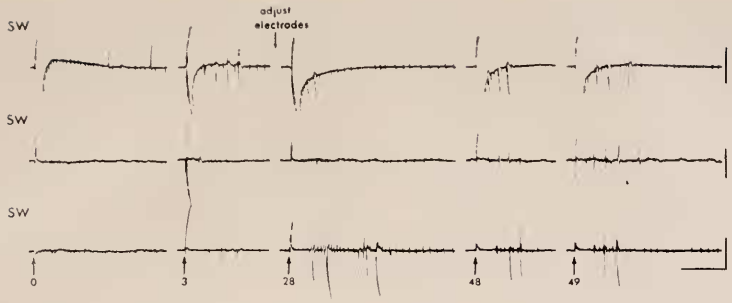


FIGURE 13. Magnesium chloride bridge experiment. Stimulus artifacts are marked by upward-pointing arrows beneath the records. The numbers beneath the arrows indicate the time (minutes) since the start of that particular portion of the experiment. See text for further details.

A distal opening response is present in *Corymorpha,* though judging from the literature this response should have been obtained much more readily and consistently than it actually was. From the electrical records obtained in the present study it is impossible to say that there is a distal opening system which is clearly separable from the TPS. Much time was spent on many animals unsuccessfully trying various methods of eliciting consistent distal opening. Fresh animals were no more responsive or consistent than those which had been in the laboratory for some time.

Nothing comparable to the slow system described by Josephson (1965) in *Tubularia* has been observed.

Neither of the experiments which might have yielded evidence for longitudinal conduction tracts in the stalk (the experiment with three electrodes arranged transversely across the stalk and Parker's experiment shown in Fig. 9) gave such evidence. However, these experiments certainly do not disprove the existence of such tracts since the suction electrodes used were non-focal and the tissue bridges in the repeat of Parker's experiment might have been too wide to reveal preferential conduction.

Kass-Simon (1972) has found that in *Hydra* bridge width is critical in determining whether or not there is differential conduction in the two directions; bridges less than 100 μ or 150 μ and wider showing no significant differences in conducting ability. In view of Parker's evidence for preferential conduction in *Corymorpha* and the fact that the bridges used in our experiments were greater than 150 μ preferential longitudinal conduction in *Corymorpha* must still be considered a strong possibility.

The failure of tetrodotoxin to block electrical activity in both *Corymorpha* and *Cordylophora* (Mackie, 1968) is certainly of interest, but beyond that little can be said in the absence of intracellular recording.

Most of the sympathomimetic drugs which were tested altered the spontaneous electrical activity of *Corymorpha.* Their most consistent effect was to change electrical activity in the proboscis to brief high-frequency bursts occurring at short intervals. In addition, overall electrical activity was increased, especially in tyramine and phenylpropanolamine. Several of the drugs had a more marked effect on isolated parts than they did on the whole animal, but it is not known whether

this result has anything to do with functional properties of the system or whether it is merely a product of reduced physical restraint of the isolated parts. Another possibility is that the drugs were able to permeate the tissues more fully through recently cut areas.

In these experiments our goal was to study the relationship between electrical activity and behavior, so we used high drug concentrations in an effort to produce clear changes. Therefore any possible role of sympathomimetic or related compounds as transmitters in *Corymorpha* remains in doubt, In view of the effects of such compounds on *Tubularia* (Parmentier and Case, 1973) and *Corymorpha* an examination of these hydroids using histochemical localization techniques (Dahl, Falck, Von Mecklenberg and Myhrberg, 1963) would be of considerable interest.

Within the limits of individual variability there was generally a change in behavior associated with altered electrical activity. In those excised hydranths which showed major alterations in electrical activity in the presence of drugs, each burst of HPs was associated with at least a partial concert, although this concert was often apparent only as a simultaneous inward twitch of all the proximal tentacles which never relaxed fully between the rapidly repeated bursts. In excised proximal tentacles to which drugs had been applied the relation between electrical activity and movment was much less clear. Sometimes a steady stream of pulses was produced without apparent movement; but even in normal isolated tentacles in sea water an obvious flexion often occurs on only the first pulse of a burst. Due to our failure to obtain a consistent distal opening response from *Corymorpha* we were unable to test the effects of drugs on the operation of this system, as was done by Parmentier and Case (1973) with *Tubularia*.

The blockage of neuromuscular transmission by excess magnesium is well established (Ross and Pantin, 1940; Bullock, 1943; Engbaek, 1952). In both cholinergic (Hubbard, Jones and Landau, 1968) and adrenergic (Kirpekar and Wakade, 1968) systems it has been suggested that the decrease in the amount of transmitter released, which is the most important factor in the blockage of neuromuscular transmission, is due to competition between Mg^{++} and Ca^{++}.

However, excess Mg^{++} apparently also can affect nerve directly. Mackie and Passano (1968), in an extensive discussion of the relation between neural and epithelial activity in hydromedusae, state that excess Mg^{++} rapidly eliminates electrical activity in systems which are known to contain nerves but in which epitheliomuscular cells may also be involved, whereas it does not prevent propagation in purely epithelial conducting systems within a comparable time span, if at all.

Before attempting to put together the results presented in this and the preceding paper into a coherent picture of the relation between electrical activity and behavior in *Corymorpha* it would appear to be worthwhile to briefly summarize the major points which such a model must accommodate. They are: 1. Most spontaneous pulses are associated with obvious contractions of the longitudinal musculature. 2. The characteristics of pulses produced by all of the spontaneous pulse systems overlap, suggesting that all of the pulses may have a common origin in epitheliomuscular cells. 3. Stalk pulses, and by analogy all other pulses associated with contraction of the longitudinal muscles, are conducted on the ectodermal side of the mesoglea. 4. Much of the behavior of *Corymorpha* which does not involve contractions of the ectodermal musculature has no recordable electrical correlate.

5. When alterations in behavior involving the ectodermal musculature are produced there is generally a corresponding change in electrical activity. 6. The processes of electrogenesis of large pulses and conduction can be separated in a bridge of tissue treated with $MgCl_2$. Also, there is conduction through perisarc-covered areas, which have a poorly developed musculature, without production of large pulses.

Points 1–5 could all be explained by hypothesizing that the activity which we have recorded consists of muscle potentials which are a concomitant of behvaior and play no role in its control. However, both the observations of Josephson and Mackie (1965) on *Tubularia* and the present studies on *Corymorpha* indicate that when either the whole animal or an excised hydranth is placed in $MgCl_2$ all visible contraction ceases considerably before any apparent change in electrical activity. Similar results were obtained when *Corymorpha* was placed in Ca^{++}-free sea water. The theories which we present below are inadequate to the extent that they fail to explain these results.

The $MgCl_2$ bridge experiments clearly separate the processes of electrogenesis of large pulses and conduction, but, in view of the apparent multiple effects of excess Mg^{++}, there are still several ways in which the results of these experiments could be interpreted. One possible explanation is that a sheet of epitheliomuscular cells can conduct without producing large pulses, either because the two processes, although occurring in the same cells, are separable, or because only a few of the cells are present (beneath the perisarc) or still capable of conducting (within a Mg^{++} treated bridge). Some form of electrical conduction would appear to be the only means by which an impulse could cross such a wide bridge of tissue at the observed speed, yet no electrical activity is recordable near the middle of the bridge. We doubt whether an electrotonically conducted pulse unrecordable at the center would be able to cross the rest of the bridge with sufficient strength to initiate a new wave of epithelial activity on the other side. A second hypothesis, which appears more likely to us, is that excess Mg^{++} has uncoupled the epitheliomuscular cells from activity in the underlying nerve net, possibly through interference with synaptic transmission between nerve cells and overlying epitheliomuscular cells. Excess Mg^{++} is known to affect neuromuscular junctions, and conduction across the high Mg^{++} area can be explained by hypothesizing that the conducting ability of the nerve continues unimpaired even though its ability to locally trigger the epitheliomuscular cells has been blocked by excess Mg^{++}.

It is obvious that many of the uncertainties in the above discussion could be removed by intracellular recordings from epitheliomuscular cells. However, all of our attempts to obtain such records have been unsuccessful (Ball, 1971), so our conclusions must be drawn from the evidence presented above. On this basis we feel that the large electrical pulses recordable from *Corymorpha* are produced in the epitheliomuscular cells, are locally propagated and provide only an incomplete picture of activity in the nervous system which is actually controlling behavior.

Many of the types of spontaneous electrical activity recorded from other hydroids are associated with obvious muscular activity and in most cases a nerve net is present running among the bases of the epitheliomuscular cells. Hypothesizing conducting epithelia as additional channels of information transfer appeared to make the explanation of the complex behavior of hydroids somewhat easier since it lessened the need for postulating different conduction pathways

and effector sensitivities for activity in a single nerve net. From studies on medusae and siphonophores (Mackie and Passano, 1968) it is clear that epithelia can serve to transmit information from one part of an animal to another and it therefore seems likely that such mechanisms should also be present in hydrozoan polyps. However, we feel that the results of our study suggest caution in accepting conducting epithelia as a means of information transfer in hydroid polyps until such time as the situation can be clarified by intracellular recording from epitheliomuscular cells.

It is a humbling fact that although our equipment was more sophisticated and we sometimes doubted his interpretations along the way, the views which we have finally reached concerning the role of the nerve net in controlling the behavior of *Corymorpha* are very similar to those expressed by G. H. Parker in the *Elementary Nervous System* in 1919.

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Summary

1. The triggered pulses (TPs) most commonly produced by *Corymorpha* in response to electrical stimulation appear similar to spontaneously occurring stalk pulses (SPs) in amplitude, waveform, and duration. Average conduction velocities in the triggered and spontaneous pulse systems are 15.7 cm/sec and 15.9 cm/sec, respectively. The TPS is non-polarized. Some animals show a relatively consistent threshold of activation; in others the threshold varies over time.

2. The behavioral response to electrical activation of the TPS varies from an almost imperceptible contraction to a vigorous inward flexion of the proximal tentacles associated with strong stalk contraction. In general, there is a clear relation between the length of a burst of electrical activity and the strength of response.

3. A small percentage (<20%) of the *Corymorpha* examined showed a distal opening response, in which all distal tentacles simultaneously flared aborally, to electrical stimulation. In a few animals this response occurred in the absence of other behavior and was associated with a single small electrical pulse; in others it was associated with contraction and a burst of TPs. Only two of more than forty animals gave a consistent distal opening response. Available data do not provide an adequate basis for establishing the DOS as a system distinct from the TPS.

4. Evidence for more than one stalk conducting system is provided by the presence of two sizes of pulses which are conducted at different velocities.

5. Experiments involving the selective destruction of endoderm or ectoderm with a glass needle were used to establish that triggered stalk pulses are conducted in the ectoderm.

6. Three electrodes lined up transversely across the stalk of a *Corymorpha* all record rather similar pulses during a spontaneous stalk burst.

7. Electrical recording from various sorts of bridge preparations provides no evidence for preferential longitudinal conduction tracts.

8. The sympathomimetric drugs tyramine, ephedrine, and phenylpropanolamine tend to cause an immediate increase in electrical activity and grouping of hydranth pulses into short high-frequency bursts. Associated with this alteration in electrical activity is an increase in the frequency of concert behavior. Another sympathomimetic drug, D-amphetamine, causes no clear change in electrical activity.

9. Tetrodotoxin (10^{-5} g/ml) has no effect on either spontaneous or triggered pulses for periods of up to 312 minutes.

10. Ca^{++}-free sea water causes whole *Corymorpha* to become motionless without apparent relaxation of the muscles. Accompanying this effect is a decrease in pulse amplitude and frequency, although pulses continue long after the animal is motionless. On return to sea water the distal tentacles are in motion within 30 seconds, followed by the proximal tentacles and proboscis within a few minutes. Electrical pulses quickly regain their full amplitude and pulse frequency begins to rise.

11. $MgCl_2$ has various effects depending on the concentration and length of exposure. The first effect is to eliminate obvious muscular activity while leaving electrical activity apparently unchanged. A few minutes later electrical activity is also reversibly eliminated. Exposure of the central portion of an isolated stalk to isotonic $MgCl_2$ while the two ends remained in sea water results in the reversible elimination of large pulses in the portion of the stalk exposed to $MgCl_2$ without interfering with conduction through the area or with the generation of large pulses at either end of the stalk. This result is interpreted as indicating that the epitheliomuscular cells are locally triggered to generate large electrical pulses by activity in the nerve net.

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